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Mini Review

Targeting Glycolysis in Proliferative Kidney Diseases

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One sentence summary: Glycolysis is a potential therapeutic target in proliferative kidney diseases, but is also important for normal function in the distal nephron.

Abstract

Glycolytic activity is increased in proliferating cells, leading to the concept that glycolysis could be a therapeutic target in cystic diseases and kidney cancer. Pre-clinical studies using the glucose analogue 2-deoxy-D-glucose have shown promise; however, inhibiting glycolysis in humans is unlikely to be without risks. While proximal tubules are predominantly aerobic, later segments are more glycolytic. Understanding exactly where and why glycolysis is important in the physiology of the distal nephron is thus crucial in predicting potential adverse effects of glycolysis inhibitors. Live imaging techniques could play an important role in the process of characterizing cellular metabolism in the functioning kidney. The goal of this mini-review is to briefly summarize recent findings on targeting glycolysis in proliferative kidney diseases, and to highlight the necessity for future research focusing on glycolysis in the healthy kidney.

Introduction

GLYCOLYSIS is the metabolic pathway that converts glucose into pyruvate, alongside this process ATP and NADH are generated. In the presence of oxygen, pyruvate is actively transported into the mitochondria and further metabolized in the tricarboxylic acid (TCA) cycle (17), which produces energy precursors for oxidative phosphorylation. In the absence of oxygen, pyruvate is converted into lactate, and glycolysis remains the main source of ATP production, so-called anaerobic glycolysis (32).

Oxygen availability in the kidney decreases gradually from the cortex to the medulla, and this is associated with a difference in metabolism between the cortical and medullary nephron segments (12). Proximal tubules in the cortex perform the bulk of fluid reabsorption and are known to be highly aerobic (54), since oxidative phosphorylation is the most efficient method to generate ATP required to drive solute transport. In contrast, distal nephron segments are much more glycolytic (15, 54). In the medulla this is probably partly explained by the lower oxygen tension, but it is also possible that glycolysis might be advantageous for some specialized functions in the cortical distal nephron segments.

Glycolysis is known to be upregulated in dividing cells in the normal development of the kidney (22), and similar findings have recently also been observed in proliferative kidney diseases. Glycolysis is therefore increasingly being investigated as a therapeutic target for cystic diseases and kidney cancer (6, 24, 42). However, given the importance of glycolysis in normal kidney function, inhibiting this process is not without risks. This mini-review will briefly summarize the experience to date of targeting glycolysis in proliferative kidney diseases, and will highlight the need for new research to better understand where and why glycolysis is important in the normal physiology of the distal nephron.

Glycolysis in proliferative kidney diseases

The "Warburg effect"

In highly proliferative cells, such as cancer cells, glycolysis is typically used as energy source even when sufficient levels of oxygen are available to perform oxidative phosphorylation. This phenomenon is called aerobic glycolysis or the "Warburg effect", initially described by Otto Warburg almost one hundred years ago (23, 50). Although not as efficient for producing ATP, glycolysis is beneficial for proliferative cells, as their energy requirements are typically low and glucose can be utilized instead for anabolic processes necessary for new cell formation (21, 23). In the following sections, we will briefly highlight two important proliferative kidney diseases, where evidence suggests glycolysis is also upregulated.

Polycystic kidney disease

Autosomal dominant polycystic kidney disease (ADPKD) is a chronic, inherited disorder that is characterized by fluid filled cysts originating in a minority of nephrons, and is caused by mutations in polycystin 1 (*Pkd1*) or 2 (*Pkd2*) (10, 14). Cell proliferation is one of the major factors contributing to cyst progression, and activation of cell proliferation signalling pathways has repeatedly been identified in ADPKD (18, 20, 38, 41, 49). In the last decade numerous studies have reported evidence of upregulated glycolysis in the pathogenesis of ADPKD (7, 24, 31, 37, 38), and this subject has recently been comprehensively reviewed by Padovano *et al.* (31). Briefly, Rowe *et al.* were one of the first to describe an alteration in metabolism in ADPKD, and that mouse embryonic fibroblasts lacking *Pkd1* rely on glycolysis as an energy source, even under aerobic conditions (38). These findings were supported by a subsequent study showing an upregulation of the glycolytic enzymes hexokinase 1 and 2 (*Hk1*, *Hk2*) and lactate dehydrogenase A (*LDHa*) in a rat model of ADPKD (37). Further *in vivo* evidence for the Warburg effect came

from a *Pkd1* inducible knock-out model, where an increased glucose uptake and conversion to lactate was observed, by following the ^{13}C-labelled forms of these molecules (7). It is important to note that not all studies have detected increased glycolysis in ADPKD (26, 51). However, it seems clear that substantial changes in metabolism occur in tubular cells during cyst development, and understanding the nature of these is likely to be crucial to fully elucidating the pathogenesis.

Kidney cancer

Consistent with the Warburg effect, glycolysis is also thought to be upregulated in clear cell renal cell carcinoma (ccRCC) (5, 33, 34, 48, 52). This is the most common type of cancer in the kidney, and is mainly caused by a mutation in the tumour suppressor gene *VHL* (29, 39). The degree of shift towards glycolysis seems to be grade dependent, and is most marked in high-grade disease (5). Upregulation of glycolysis is associated with a simultaneous decrease in expression of TCA cycle genes, but an increase in genes involved in glutamine transport and fatty acid synthesis (5). These findings are likely explained by studies performed in *VHL*-deficient ccRCC cells, which showed that the TCA acts in reverse (so-called reductive carboxylation), using glutamine to generate citrate, which can then be exported to the cytosol to increase the production of macromolecules needed for cell proliferation (13). Interestingly, there is now evidence that his phenomenon might also occur in ADKPD (35), suggesting overlap in the disease mechanisms.

Glycolysis as treatment target

Given that glycolysis seems to be a crucial process in cell proliferation, it follows that inhibiting glycolysis might be beneficial in treating diseases like ADPKD and ccRCC, and numerous studies are now investigating this possibility. Aerobic glycolysis can potentially be inhibited on

many levels (reviewed in (27, 31, 42, 44)), including: the uptake of glucose into the cell by glucose transporters; the breakdown of glucose to glucose-6-phosphate by hexokinase; the formation of pyruvate by pyruvate kinase; and the conversion of pyruvate to lactate by LDH. Furthermore, besides glycolysis, the increased uptake of glutamine can also be targeted to inhibit reductive carboxylation (13, 35). However, the strategy that has received most attention is direct inhibition of glycolysis with the glucose analogue 2-deoxy-D-glucose (2DG).

2-deoxy-D-glucose

2DG is taken up by cells and phosphorylated by hexokinase, but is not further catabolized in the glycolysis pathway (53). Although widely used in cancer biology (55), it has not yet been extensively tested in pre-clinical ccRCC models. However, a recent study using primary cell cultures from low- and high-grade ccRCC showed that 2DG significantly decreased lactate secretion and ATP levels only in the latter (2). This suggests that the usage of glycolysis inhibition as a therapy might need to be tailored to individual patients, depending on information acquired from tissue histology.

Meanwhile, 2DG has also been tested in various *in vitro* and *in vivo* studies of ADPKD. Administration of 2DG significantly retarded cyst progression in two different rodent models (7, 37). Furthermore, a low dose combinational treatment of 2DG with metformin (normally used for the treatment of type 2 diabetes mellitus) was shown to inhibit cell proliferation in human-derived cystic kidney epithelial cells (56). This combination treatment was also tested in a miniature pig model, and was shown to markedly reduce renal cyst formation and protect renal function (19).

Overall, early studies have suggested that 2DG has some promise as a therapy for proliferative kidney disease. However, glycolysis is a fundamental and widespread metabolic process in living

organisms, and glucose depletion in the blood (hypoglycaemia) is known to produce serious adverse effects (43), including in the nervous, immune and respiratory systems (9, 25). Thus, intentionally inhibiting glycolysis *in vivo* brings significant potential risks, and careful attention needs to be given to safety issues.

Safety issues with blocking glycolysis in vivo

The use of 2DG has been reported in phase I clinical trials of different types of tumours and generally it seems to be surprisingly well tolerated (36, 43, 45). Therefore, it should be possible to implement its use in clinical trials of ADPKD patients (reviewed in (24)). However, the safety profile of this compound, together with the appropriate dose, needs to be more carefully determined. While some animal studies report no degree of toxicity over prolonged periods of administration of 2DG (7, 30), others have described severe adverse cardiac effects (28, 46). Moreover, cardiac abnormalities, together with disorientation, tiredness, and excessive sweating have also been observed in the human clinical trials (36, 45).

Finding a treatment regime that produces sufficient blockade of glycolysis in proliferating cells to substantially retard disease progression, but without adversely altering metabolism in healthy tissues, represents a major logistical challenge. This concept is particularly pertinent to the kidney, where levels of glycolysis are normally quite high in the medulla (Figure 1A). Moreover, besides producing ATP, glucose is also important for other metabolic processes, such as the generation of NAD(P)H via the pentose phosphate pathway. Indeed, we recently found that administration of 2-DG acutely decreases mitochondrial and cytosolic NAD(P)H signals in proximal tubules (4).

Thus, while inhibiting glycolysis could be of benefit in retarding the progression of kidney cysts and tumours, side effects might arise, partly due to a loss of normal specialized functions in distal nephron segments. To understand the nature and magnitude of these effects, it will first be necessary to pin-point which cell types and transport processes are particularly dependent on glycolysis. This in turn will necessitate the development of new techniques to study cellular metabolism in intact functioning kidney tissue.

Glycolysis in the distal nephron segments

Within the distal nephron, previous studies have suggested that the collecting duct (CD) is the site with the highest glycolytic activity. CDs express more glycolytic enzymes than other segments (15), generate more lactate (1), and maintain ATP levels better when aerobic respiration is inhibited (47). Unlike other segments, CDs comprise of two very different cell types interspersed with each other, which have very distinct transport functions. Principal cells (PCs) are responsible for sodium and water homeostasis, whereas intercalated cells (ICs) maintain an acid-base balance (8). In parallel with transport functions, it is likely that PCs and ICs also display differences in metabolism. However, relatively little is known about this, due to the technical challenges of isolating cells in an appropriate state to perform standard metabolic assays. Thus, most studies have historically considered the CD as a single entity, and it therefore remains unclear which cell type is predominantly responsible for the overall glycolytic phenotype, and also which specific transport processes might be dependent on glycolysis.

Morphological studies such as electron microscopy reveal major differences in ultrastructure between cells in the CD. Of relevance to metabolism, the density of mitochondria is much higher in ICs than in PCs (3) (Figure 1B), perhaps hinting that the latter might be more glycolytic.

However, mitochondria have important functions other than generating ATP, so it does not necessarily follow that ICs generate more ATP via oxidative phosphorylation.

The development of high-resolution live imaging techniques like multiphoton microscopy provides a potential new avenue to study cellular metabolism in living animals (16, 40). Using this approach, it has recently been described that cells in the rat CD display striking differences in the uptake of mitochondrial dyes, hinting at the existence of major differences in metabolism between PCs and ICs (11). We have also observed similar signal patterns in mice (Figure 1C), and are currently utilizing multiphoton microscopy - in parallel with other imaging techniques - to try to further understand the significance of these findings, and what they might tell us about the nature of metabolism along the CD. We envisage that such an approach could finally shed some light on the relationship between glycolysis and transport function in the distal nephron, understanding of which could be crucial in anticipating adverse effects of glycolysis inhibitors.

Conclusion

Glycolysis is upregulated in proliferating cells and has thus been identified as a novel therapeutic target in ADPKD and kidney cancer. Pre-clinical studies with blocking agents such as 2DG have shown some promise, but inhibiting glucose metabolism in humans is unlikely to be without risk. In particular, glycolytic activity is thought to be high in segments like the CD. It is therefore crucial to further elucidate where and why glycolysis is important in the nephron, and live cell imaging techniques could play an important role in this process.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

Author contributions

S.G. and M.P. prepared the figures; S.G. drafted manuscript; A.M.H. edited and revised manuscript; A.M.H. approved final version of manuscript.

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Figure legends

Figure 1: Glycolysis in the kidney and heterogeneity of the collecting duct. (A) The oxygen gradient in the kidney decreases gradually from the cortex to the medulla, and glycolytic activity is higher in the latter. (B) Electron microscopy of a cortical collecting duct (CD), showing that intercalated cells (ICs) have a much higher mitochondrial density than neighboring principal cells (PCs). (C) *In vivo* multiphoton imaging of a mouse kidney reveals a heterogeneous uptake along the cortical CD of the voltage dependent mitochondrial dye Tetramethylrhodamine (TMRM) (red). TMRM signal intensity provides a readout of mitochondrial energization within living cells, which is dependent on respiratory chain activity. Scale bar = 5 µm (A) and 10 µm (C). Schematic image was produced using scientific illustration toolkits ɪrom Motifolio.

Figure 1